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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/622,240	07/18/2003	George Tzertzinis	NEB-208/9-US	3580

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EXAMINER	
POPA, ILEANA	

ART UNIT	PAPER NUMBER
1633	

MAIL DATE	DELIVERY MODE
07/12/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 10/622,240	**Applicant(s)** TZERTZINIS ET AL.	
	Examiner Ileana Popa	**Art Unit** 1633	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 11 June 2007 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires 6 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☒ The Notice of Appeal was filed on *11 June 2007*. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☒ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: *1,2,5-7,9,12-14,16-18,20 and 47*.
   Claim(s) withdrawn from consideration: *8,10,15,19 and 21-46*.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☐ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: _____.
12. ☐ Note the attached Information Disclosure Statement(s). (PTO/SB/08) Paper No(s). _____
13. ☒ Other: see continuation sheet.

Ileana Popa

Claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Yang et al. in view of Gross et al. (Nucleic Acids Research, 1987, 15: 431-442) for the reasons of record set forth in the prior Office action.

Applicant argues that:

(i) While the claimed enzyme to subtrate ratio is 0.25:1, Yang et al. utilizes a ratio of about 0.001:1 and teach away from using increased amounts of enzyme due to exhaustive cleavage that leads to products averaging 12-15 bp in length; therefore, Yang et al. teach a method of performing limited digestion; Gross et al. cleave substrate RNA that is not compatible with the substrate of Yang et al. because the substrate before cleavage is a very short dsRNA of 12 bp maximum, while the minimum size of the cleavag eptoduct of long dsRNA in Yang et al. is 12 bp; it is not possible to deduce enzyme to substrate ratios from the teachings of Gross et al.;

(ii) Gross et al. do not suggest cleaving large dsRNAs into fragments of 15-30 bp;

(iii) In contrast to the specific cleavage described by Gross et al., Yang et al. report the lack of sequence specificity in substrate recognition and cleavage by RNaseIII; any suggestion of specific cleavage sites in Gross et al. teaches away from the cleavage pattern demonstrated by Applicant in Fig. 4 of the specification;

(iv) It cannot be determined from the teachings of Yang et al. or Gross et al. whether RNase III digestion products overlap;

(v) The size fractionation of the cleavage products by Yang et al. to remove large dsRNA fragments likely affects the distribution of the cleaved fragments. There is no suggestion in the art as to how short ssRNA that form the hairpin substrate of Gross et al. interact with RNaseIII and manganese and how this compare to the cleavage of large dsRNA in the presence of divalent metal cations;

(vi) In contrast to Yang et al., who teach how to avoid exhaustive digestion, Gross et al. do not adress the problem of exhaustive digestion, but rather a different problem, i.e., how to introduce, instead of reduce, cleavage sites in very short region of short dsRNA and conseqeuntly, Gross et al. teach away from Yang et al.;

(vii) In contrast to Yang et al., Applicant claims an improved method of preparing dsRNAs of defined size (15-30 bp) by using an RNase III to substrate ratio of 0.25:1 and a reaction buffer containing divalent transition metals, such as manganese. Applicant surprisingly discovered that the use of divalent transiiton metals in place of magnesium resulted in an enriched population of cleavage overlapping fragments of 14-30 bp that did not require the size fractionation of Yang et al. to obtain a preparation of dsRNA with defined size;

(viii) The routine experimentation suggested by Yang et al. is to use lower enzyme to substrate ratio, which teaches away from the relatively high ratio required by the claimed method. Gross et al. do not provide a ratio and are silent regarding the effect of varying enzyme concentration.

Applicant's arguments are acknowledged, however, the rejection is maintained for the following reasons:

The argument that Yang et al., by disclosing lower enzyme to substrate ratios to avoid exhaustive cleavage, teach away from using increased amounts of enzyme, as required by the instant claims, is not found persuasive because it is the combination of reference that makes the claimed invention obvious. Young et al. teach a method of producing a plurality of siRNAs by cleaving dsRNAs with RNase III. With respect to the argument that Yang et al. do not teach ovelapping fragments, it is noted that producing ovelapping fragments is an inherent propery of RNase III, which unspecifically cleaves the RNA and therefore necessarilly produce overlapping fragments following digestion (therefore, the argument that it cannot be determined, from the teachings Yang et al. or Gross et al. whether the RNase III digestion products overlap is not found persuasive). Although Yang et al. do not teach managanese, the prior art teaches that RNase III can be used in a buffer containing manganese instead of magnesium. Among others, Gross et al. teach that RNase III can use manganese for catalytic activity. It is noted that the substrate of Gross et al. before cleavage is not a 12 bp dsRNA as Applicant submits; the RNase III substrate for both magnesium-containing and manganese-containing reactions is a 141 base long RNA, wherein primary sites located at stretches of base pairing in the RNA are efficiently cleaved in the presence of borh magnesium and manganese and wherein additional sites are cleaved only in the presence of manganese and wherein the secondary sites are located in short dsRNA stretches located at the bottom or in the middle of a ds stem region (p. 439, second full paragraph, p. 441, first full paragraph, Fig. 3 and 4). Therefore, the full 141 base long RNA, and not a short dsRNA of maximum12 bp, is used as substrate, similar to the teachings of Young et al., who teach cleavage of long RNAs (i.e., the substrates are compatible). Therefore, Gross et al. teach that cleavage by E. coli RNase III can be rendered more efficient by exchanging magnesium with manganese. The fact that Gross et al. do not teach cleaving the RNA into fragments of 15-30 bp is irrelevant because the references was cited for teaching other claim limitaitons (i.e., manganese) (see the prior Office actions). The argument that Gross et al. teach away from the instant invention because they disclose specfic cleavage by RNase III is not found persuasive because Gross et al. teach that the secondary sites are identical to the primary sites recognized by the E. coli RNase III in the presence of magnesium (p. 432, first paragraph), i.e., the cleavage at both primary and secondary sites is unspecific. Applicant's argument that fractionation of the cleavage products in the method of Young et al. affects the distribution of the cleaved fragments is just an argument; beside an argument, Applicant did not provide any evidence indicatiung that indeed this is the case. the argument that Gross et al. teach away from Young et al. because they teach how to introduce, instead of reduce, cleavage sites in very short region of short dsRNA is also not found persuasive for the reasons stated above. It is also noted that Gross et al. teach that a complete digestion of their RNA substrate has not been obtained, regardless of the presence of magnesium or manganese (p. 439, p. 441). With respect to the claimed improved method, it is noted that this the method taught by Young et al. and Gross et al. would necessarilly result in the claimed improved results. With respect to the argument that the routine experimentation suggested by Yang et al. is to use lower enzyme to substrate ratio, which teaches away from the relatively high ratio required by the claimed method, it is noted that this is pertaining to the use of a buffer containing magnesium and not manganese. One of skill in the art would readilly recognize that changing the divalent cation would necessarilly change the conditions for the enzymatic reaction and would recognize the necessity to find the redifining the optimum conditions when the reaction is performed in the presence of manganese.

Applicant argues that lines 3, 6, and 9 describing 21 bp siRNA marked by an arrow in in Fig. 1B of Young et al. represent size markers and not esiRNA because in paragraph 0053 Young et al. teach that only siRNA used for gene silencing is called esiRNA. In response to this argument, it is noted that Young et al. disclose preparation of of a heterogenous siRNA (and not esiRNA) population that could target multiple sites on RNA from dsRNA by digestion with RNase III, wherein, for simplicity, the heterogenous siRNA population is named esiRNA (i.e., endonucelase-prepared siRNA) (see paragraphs 0005 and 0053). Therefore, it cannnot be assumed, as Appliucant argues, that the 21 bp siRNA marked by an arrow (lanes 3, 6, and 9 of Fig. 1B) are size markers obtained by chemical synthesis. At most; one of skill in the art would assume that it is uncelar whether these siRNAs are the product of cleavage or markers.

For all the reasons above, and for the reasons set forth in the prior Office actions, the rejection is maintained.